High-Density Lipoprotein Cholesterol (HDL-C) Content Assay Kit

Note: The reagents have been changed, please be aware of and follow this instruction strictly.

Operation Equipment: Spectrophotometer

Catalog Number: BC5320

Size: 50T/48S

Components:

Extract I: Isopropyl alcohol 60 mL ×1. Required but not provided. Store at 2-8℃. A 30 mL brown empty bottle is provided in the kit, which is only used for packaging. Please mark the name of the reagent yourself.

Extract II A: Liquid 7.5 mL×1. Store at 2-8℃.

Extract II B: Liquid 7.5 mL×1. Store at 2-8℃. According to the required amount of the experiment, according to the ratio of extract II A: extract II B =100 μL: 100 μL (1T amount), mix well, and prepare before use.

Reagent I: Liquid 5 mL×1. Store at 2-8℃.

Reagent II: Liquid 70 mL×1. Store at 2-8℃.

Reagent III: Liquid 0.45 mL×1. Store at 2-8℃.

Reagent IV: Liquid 0.07 mL×1. Store at 2-8℃.The liquid is placed in the EP tube inside the reagent bottle.

Standard Solution: Powder ×1, 10 mg cholesterol. Store at 2-8℃. Add 517 μL of Extract I before use and shake to dissolve. The cholesterol standard solution of 50 μmol/mL could be stored at 2-8℃ for four weeks.

Working Solution: Prepare the working solution according to the ratio of Reagent I: Reagent II: Reagent III: Reagent IV=0.63 mL: 8.37mL: 60 μL: 9 μL (a total of 9.069 mL, about 10T) according to the sample volume, and prepare the working solution as it is used.

Product Description

High-density lipoprotein (HDL) is a lipoprotein that contains a small amount of cholesterol and carries cholesterol away from body cells and tissues. High-density lipoprotein cholesterol (HDL-C) concentrations negatively correlate with the incidence of atherosclerosis and coronary heart disease. Therefore, accurate and precise measurements of patients' HDL-C concentrations are necessary to appropriately identify individuals with atherosclerosis, coronary heart disease and hypertension.

Cholesterol is specifically dissociated by one surfactant from HDL. Esterase can catalyze the hydrolysis of cholesterol ester to produce free cholesterol (FC) and free fatty acid (FFA), thus transforming cholesterol ester into FC; Furthermore, cholesterol oxidase can catalyze FC to form 4-cholesterone and H_2O_2; Finally, peroxidase can catalyze the oxidation of 4-aminoantipyrine and phenyl amines by H_2O_2 to form purple quinones. It has a characteristic absorption peak at 546 nm, and its color depth is directly proportional to cholesterol content.

Reagents and Equipment Required but Not Provided.

Spectrophotometer, balance, table centrifuge, constant temperature incubator/water bath, 1mL glass cuvette, pipette, mortar/homogenizer/cell ultrasonic crusher, ice, distilled water, **isopropyl alcohol**.

Procedure

I. Sample preparation:

1. Tissue: according to tissue weight (g): Extract I volume (mL) is 1:5-10. (It is recommended that add 1 mL of Extract I to 0.1 g tissue). Homogenate in ice bath, then centrifuge at 10000 g for 10 minutes at 4°C. Take the supernatant for test.

2. Bacteria/cells: according to the number of bacteria/cells (10^4): the volume of Extract I (mL) is 500~1000:1. It is suggest that add 1 mL of Extract I to 500 million of cells. Breaking bacteria/cells by ultrasonic wave in ice bath (power 300W, ultrasonic 2s, interval 3s, total time 3 min). Centrifuge at 10000 g 4°C for 10 minutes. Take the supernatant on ice for test.

3. Serum (plasma) or other liquid samples: detect directly. Centrifuge before detecting if there are precipitation in the liquid.

II. Determination Procedure

1. Preheat the spectrophotometer for more than 30 minutes, adjust the wavelength to 546 nm and set zero with distilled water.

2. Standard working solution: Dilute 50 µmol/mL standard solution with **Extract I** to 2, 1.25, 0.625, 0.3125, 0.15625, 0.078125 µmol/mL for standby.

3. Operation table:

Reagent (µL)	Test tube (A_T)	Standard tube (A_S)	Blank tube (A_B)
Sample	200	-	-
Standard	-	200	-
Extract II	200	200	-
Mix well. React at room temperature for 10 minutes. Centrifuge at 3000rpm for 15 minutes at room temperature and take the supernatant.			
Supernatant	100	100	-
Extract I	-	-	100
Working Solution	900	900	900
Mix thoroughly. React at 37°C for one hour. Measure the absorption at 546 nm and record as A_T, A_S, A_B. Calculate $\Delta A_T = A_T - A_B$, $\Delta A_S = A_S - A_B$. Blank tube and standard curve only need to test once or twice. Note: If the sample is a liquid sample such as serum (plasma), it is necessary to add a 'serum (plasma) blank tube' - i.e., the extraction solution I (isopropanol) in the blank tube is replaced with distilled water for the experiment, and the $\Delta A_T = A_T - A_B$（serum (plasma) blank）, while the assay in the standard tube and the calculation of ΔA standard remain unchanged.			

III. Calculation of HDL-C Content:

1. Standard curve

Taking the concentration of each standard solution as the x-axis and its corresponding ΔA_S as the y-axis, draw a standard curve to get the standard equation $y = kx + b$, and bring ΔA_T into the equation to get x (μmol/mL).

2. Calculation

1) Serum (plasma) or other liquid samples: HDL-C content (μmol/dL) $= x \times 100$

2) Protein concentration: HDL-C content (μmol/mg prot) $= x \times V_E \div (Cpr \times V_E) = x \div Cpr$

3) Sample weight: HDL-C content (μmol/g weight) $= x \times V_E \div W = x \div W$

4) Bacteria/cells number: HDL-C content (μmol/10^4 cell) $= x \times V_E \div N = x \div N$

   100: Unit conversion factor, 1 dL=100 mL;

   V_E: Added Extract I volume, 1 mL;

   W: Sample weight, g;

   N: The number of bacteria/cells, in tens of thousands of units;

   Cpr: The concentration of protein, mg/mL;

Note:

1. If samples ΔA_T is too high, it is suggested that the samples should be diluted with multiple times of Extract I. Sample supernatant volume could be increased if samples ΔA_T is too low. And modify the calculation formula.

2. The protein concentration can be detected in another tissue.

Experimental example:

1. Take 0.2 mL of human serum, operate according to the determination steps, calculate $\Delta A_T = A_T - A_B = 0.216 - 0.027 = 0.189$. Bring the result into the standard curve $y=0.7591x-0.0528$ and calculate $x=0.319$. The result is calculated according to liquid volume:

   HDL-C content (μmol/dL) $= x \times 100 = 0.319 \times 100 = 31.854$ μmol/dL.

2. Take 0.1g mice liver, add 1 mL of Extract I, grind the homogenate with ice bath. Then operate according to the determination steps, calculate $\Delta A_T = A_T - A_B = 0.082 - 0.027 = 0.055$. Bring the result into the standard curve $y=0.7591x-0.0528$ and calculate $x=0.142$. The result is calculated according to sample weight:

   HDL-C content (μmol/g weight) $= x \div W = 0.142 \div 0.1 = 1.420$ μmol/g weight.

References：

[1] Warnick G R, Nauck M, Rifai N I. Evolution of Methods for Measurement of HDL-Cholesterol: From Ultracentrifugation to Homogeneous Assays[J]. Clinical Chemistry, 2001, 47(9):1579-1596.

[2] Yan S, Lin Q. Methods for Detection of High-Density Lipoprotein Cholesterol and its Standardization[J]. Chinese Journal of Laboratory Medicine, 1998, 21(1): 19-22.

Related products：

BC0590/BC0595   Free fatty Acids(FFA) Content Assay Kit

BC0750/BC0755   Acetaldehyde Dehydrogenase(ALDH) Activity Assay Kit

BC1890/BC1895   Free Cholestenone(FC) Content Assay Kit

BC1980/BC1985   Total Cholestenone(TC) Content Assay Kit